Supplemental

Protein Production and Purification

Murine 1G8 anti-PSCA intact antibody and humanized anti-PSCA A11 affinity matured minibody derived from 1G8 were produced and purified as previously described (*1–3*). Soluble human PSCA antigen was generated as a PSCA-murine Fc (PSCA-mFc) fusion protein in mammalian NS0 cells transfected with a pEE12 vector (Lonza). Supernatant was loaded onto a Protein A column in PBS using an ÄKTA Purifier (GE Healthcare). Bound protein was eluted with PBS containing 9% (v/v) 0.1 M Citrate, pH 2.1. Eluted fractions were neutralized upon collection with 5% (v/v) 1 M Tris, pH 8.0. Fractions containing PSCA antigen were identified by SDS-PAGE and western blot, combined, dialyzed into PBS (Slide-A-Lyzer, Pierce), concentrated using VivaSpin centrifugal concentrator (Vivascience, MWCO: 30,000), and stored at 4°C.

Cell Lines and Xenografts

22Rv1 (PSCA-negative) cell lines were obtained from American Type Culture Collection. 22Rv1 cells were retrovirally transfected with PSCA (22Rv1×PSCA) as previously described (*4*). 22Rv1 and 22Rv1×PSCA cell lines were grown in RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin. 22Rv1 and 22Rv1×PSCA tumors were implanted bilaterally by injecting 1×10^6 cells in 1:1 PBS:Matrigel (BD Bioscience) subcutaneously into the contralateral shoulders of male nude mice and were allowed to grow for two weeks before imaging. LAPC-9 tumors were passaged surgically between male SCID mice as previously described, and were allowed to grow for 3-4 weeks (*5*). All animal experiments were conducted in compliance with a protocol approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles.

Cell Binding

PSCA expression per cell for LAPC-9, 22Rv1, and 22Rv1×PSCA was determined by quantitative flow cytometry. LAPC-9 tumors were reduced to single cell suspensions by incubation in 1 mg/mL collagenase IV (Sigma) in HBSS supplemented with 3 mM

$CaCl_2$ for 1 hour at 37°C and passaged through 18 and 24 gauge needles followed by a 70μm cell strainer (BD Biosciences). 5×10^5 22Rv1, 22Rv1$\times$PSCA, and LAPC-9 cells were incubated with 250μL of 16μg/mL ($\sim$100nM) 1G8 mouse anti-PSCA antibody followed by 8μg/mL ($\sim$50nM) Dylight-649 conjugated anti-mouse-Fc_γ secondary antibody (Jackson ImmunoResearch). Following secondary antibody incubation, samples were incubated with Alexa-488 anti-PSMA antibody (FOLH1, BioLegend) and stained with 7-AAD (BD Biosciences), as per the manufacturers' instructions, to allow gating for viable epithelial cells in the digested LAPC-9 tumors. To quantitate the mean number of PSCA antigens expressed per cell, calibration beads (QIFIKIT, Dako) were used as per the manufacturer's instructions. Acquisition was performed with an LSRII flow cytometer (BD Biosciences) and analysis was performed with FlowJo 9.3.2.

The apparent affinity of the A11 minibody was measured by flow cytometry using 22Rv1 and 22Rv1$\times$PSCA cells. 10^5 22Rv1 and 22Rv1$\times$PSCA cells were each incubated in 400μL of 1%FBS/PBS at concentrations of A11 minibody ranging from 0 to 512nM for 1 hour at 4°C in triplicate. The samples were then washed and incubated with 8μg/mL ($\sim$50nM) Dylight-649 anti-human-Fcγ (Jackson ImmunoResearch) secondary antibody for 1 hour. The apparent affinity of the minibody on 22Rv1$\times$PSCA cells was determined by fitting the mean fluorescence intensity of each sample to a one-site saturation binding model.

Radiolabeling

Anti-PSCA A11 minibody was conjugated to the chelator desferrioxamine (DFO-A11) by incubation with a 3-fold molar excess of p-isothiocyanatobenzyl-desferrioxamine (SCN-DFO, Macrocyclics) for 30 minutes (pH 9.0, 37°C) followed by removal of free DFO and buffer exchange into PBS using a PD-10 column (GE Healthcare) and concentration using a VivaSpin centrifugal concentrator (Vivascience, MWCO: 30,000) (*6*). ^{89}Zr-DFO-A11 (^{89}Zr-A11) was prepared by incubating DFO-A11 with $\sim$150MBq/mg ($\sim$4μCi/μg) ^{89}Zr-Oxalate (Washington University in St. Louis) at pH 7.0 for 1 hour at room temperature (*6*). ^{124}I-labeled-A11 minibody (^{124}I-A11) was prepared using tubes pre-coated with Iodogen reagent (Pierce) as previously described using $\sim$150MBq/mg ($\sim$4μCi/μg) ^{124}I-NaI in 0.02 M NaOH provided by IBA Molecular (*7*).

Both ^{124}I-A11 and ^{89}Zr-A11 were purified using microcentrifuge size exclusion columns (Micro Bio-Spin, Bio-Rad) and radiochemical purity was assessed by Instant Thin Layer Chromatography (ITLC, Biodex Medical Systems) using either normal saline (^{124}I-A11) or 20 mM Citrate pH 5.0 (^{89}Zr-A11) as the mobile phase. The immunoreactive fraction of both ^{124}I and ^{89}Zr-labeled A11 was measured as the percentage of cell

bound antibody when a trace amount of antibody (<0.5 pMol) was incubated with excess 22Rv1×PSCA cells (∼50×10^6 cells, bearing >200 pMol PSCA antigens) in 1 mL of 1%FBS/PBS for 1 hour at room temperature. 22Rv1 cells were used as a negative control. The stability of both ^{124}I-A11 and ^{89}Zr-A11 was measured *in vitro* by incubation in 1%FBS/PBS and mouse serum for 44 hours at 37°C followed by remeasuring the radiochemical purity by ITLC.

Affinity Studies

The apparent affinity of unmodified A11, DFO-A11, and A11 conjugated to non-radioactive iodine, were measured by quartz crystal microbalance using an Attana Cell A200. Human PSCA-mFc antigen (40μg/mL) was immobilized on a LNB-carboxyl sensor chip by amine coupling. Binding experiments were performed in HBS 0.005% Tween 20 (25μL/min, 22°C). Five serial dilutions (320-20 nM) of each construct were run in triplicate in random order. The chip was regenerated using 0.1 M Glycine pH 2.5 between each sample. Buffer injections were performed prior to each sample injection to use as a reference in integrated Attester Evaluation software where the binding curves were fit using a mass transport limited binding model.

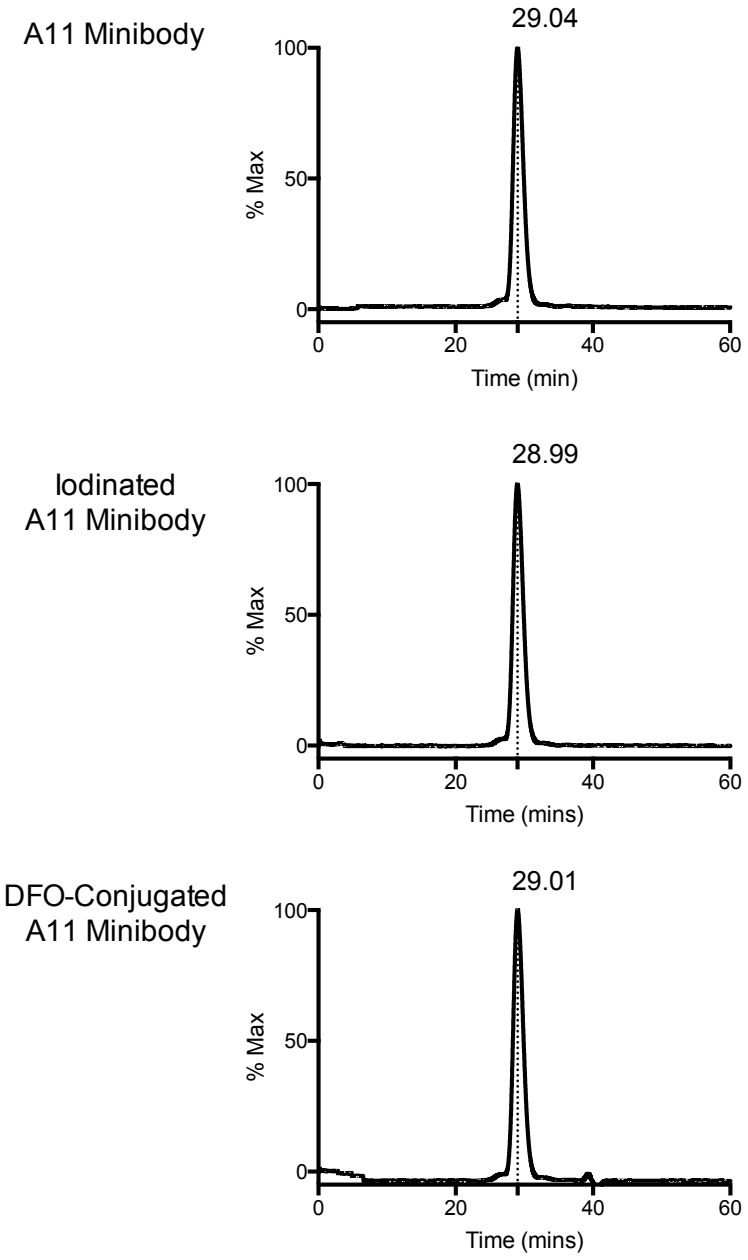
***In vitro* Cellular Antibody Uptake**

22Rv1 and 22Rv1×PSCA cells were grown to 75% confluence in 12 well plates for 2 days. The cells were then pre-incubated for 1 hour at 4°C in growth media containing 5μg/mL (∼60nM) of either ^{124}I-A11 or ^{89}Zr-A11. After the pre-incubation, cells other than the zero time point were warmed to 37°C for the remainder of the experiment. At each time point (0-44 hours), the supernatant and washes (1%FBS/PBS), the cell bound fraction (150 mM NaCl, 0.1 M Glycine pH 2.5), and the internalized fraction (PBS, 10 mM Tris, 0.5% SDS, pH 7.4) were each collected and activity was measured with a gamma counter (WIZARD3, Perkin Elmer). The experiment was performed in triplicate and data were normalized to the total activity in each well.

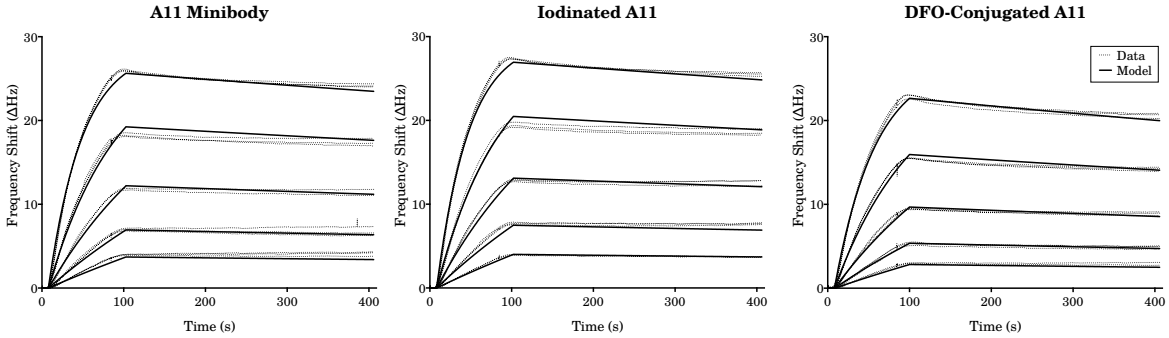
Biodistribution

Following the final imaging time point (44 hours), mice were sacrificed and dissected. Blood, tumors, and other organs were then weighed and gamma-counted. The results

were converted to %ID/g using a standard consisting of 1% of the injected dose made at the time of radiolabeling.

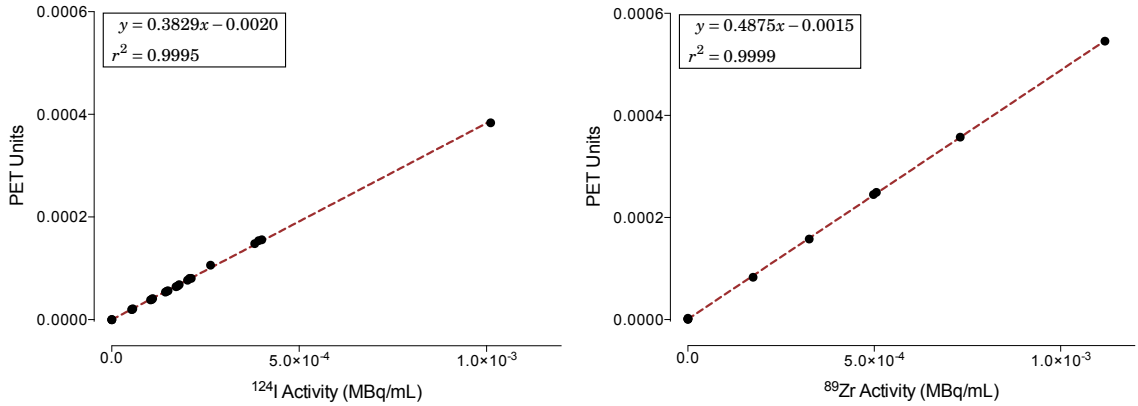


Supplemental Figure 1: Comparison of unconjugated A11, Iodinated A11, and DFO conjugated A11 by size exclusion chromatography shows no significant change in size or multimerization following conjugation.

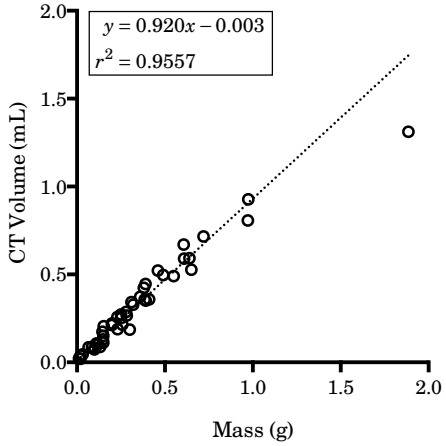


	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	B_{Max}	K_D (nM)
A11 Minibody	7.43×10^{4}	2.90×10^{-4}	28.90	3.91
Iodinated A11	7.83×10^{4}	2.68×10^{-4}	30.02	3.43
DFO-Conjugated A11	6.09×10^{4}	4.11×10^{-4}	27.57	6.75

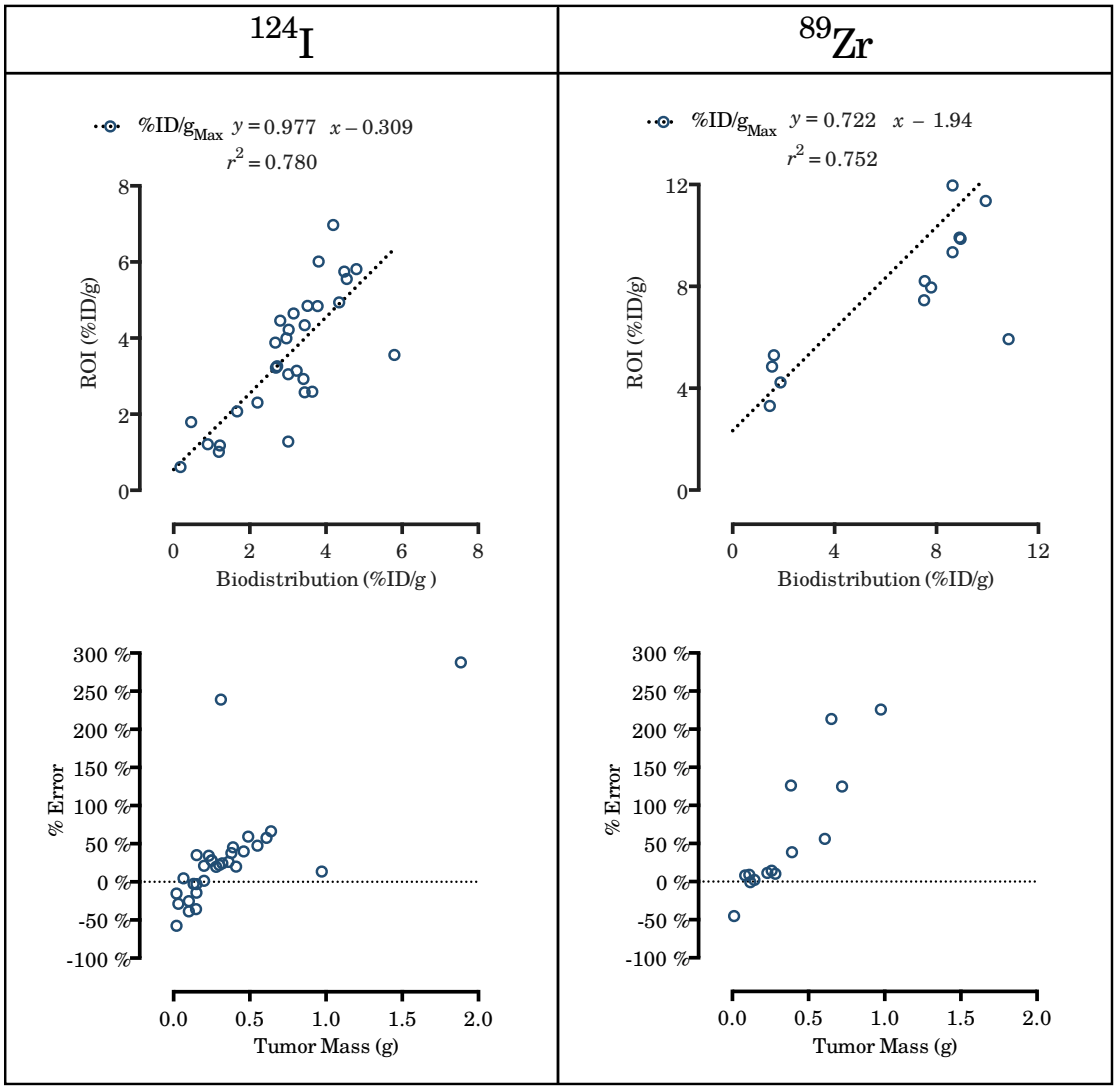
Supplemental Figure 2: Affinity Measurements of A11 minibody, Iodinated A11, and DFO-conjugated A11 binding to immobilized PSCA antigen as measured by quartz crystal microbalance. Bold line is the mass transport limited binding model fit from $n = 3$ measurements at each concentration (320–20 nM, shown as dotted lines).



Supplemental Figure 3: Inveon microPET Calibration for ^{124}I and ^{89}Zr. MicroPET scanning of ^{124}I and ^{89}Zr at a variety of concentrations shows a linear microPET scanner response. The slope of each linear regression has units of PET Units/MBq/mL and was used as the cylinder factor for the respective radionuclide.



Supplemental Figure 4: Comparison of tumor masses and CT volumes approximated with ellipsoidal ROIs shows excellent correspondence. Linear fitting was weighted by $1/y^2$ as volume error is proportional to tumor size.



Supplemental Figure 5: Quantification of tumor activity by the maximum voxel in the tumor (%ID/g_{max}) exhibits a strong partial volume effect and yields noisier data than with the mean value or partial volume corrected mean value.

Bibliography

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